

AD \_\_\_\_\_

Award Number: MIPR 7JBJYXM8914

TITLE: Novel Recruitment Techniques for a Study of Culture-Specific Diet, Metabolic Variability, and Breast Cancer Risk in African-American Women

PRINCIPAL INVESTIGATOR: Christine B. Ambrosone, Ph.D.  
Fred F. Kadlubar, Ph.D.

CONTRACTING ORGANIZATION: National Center for Toxicological  
Research  
Jefferson, Arkansas 72079-9502

REPORT DATE: August 2001

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

# REPORT DOCUMENTATION PAGE

Form Approved  
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

<b>1. AGENCY USE ONLY (Leave blank)</b>		<b>2. REPORT DATE</b> August 2001	<b>3. REPORT TYPE AND DATES COVERED</b> Annual (1 Jul 00 - 1 Jul 01)	
<b>4. TITLE AND SUBTITLE</b> Novel Recruitment Techniques for a Study of Culture-Specific Diet, Metabolic Variability, and Breast Cancer Risk in African-American Women			<b>5. FUNDING NUMBERS</b> MIPR 7JBjYXM8914	
<b>6. AUTHOR(S)</b> Christine B. Ambrosone, Ph.D. Fred F. Kadlubar, Ph.D.				
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b>  National Center for Toxicological Research Jefferson, Arkansas 72079 -9502-  E-Mail: <a href="mailto:fkadlubar@nctr.fda.gov">fkadlubar@nctr.fda.gov</a> <a href="mailto:Christine.ambrosone@mssm.edu">Christine.ambrosone@mssm.edu</a>			<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>	
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b>  U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			<b>10. SPONSORING / MONITORING AGENCY REPORT NUMBER</b>	
<b>11. SUPPLEMENTARY NOTES</b>				
<b>12a. DISTRIBUTION / AVAILABILITY STATEMENT</b> Approved for Public Release; Distribution Unlimited				<b>12b. DISTRIBUTION CODE</b>
<b>13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information)</b> African-American women have younger breast cancer diagnosis, more aggressive tumors, and higher mortality rates than Caucasians. Ascertaining reasons for disparities is hampered due to the difficulty in enrolling African-Americans into research studies. This study was designed to develop a novel method of recruitment, and to collect pilot data on risk factors that could, in part, explain racial differences in breast cancer. Cases and controls are sent an introductory postcard by a breast cancer survivor from their town or county, with her photograph on it, followed by a telephone call from that recruiter. To date, the recruitment methodology is established, and 75 African-American cases and 30 controls have been interviewed, with response rates of 63% and 56%, respectively. In a previous study, rates were 37% and 30%. Interviews have been conducted and are ongoing, data has been double-entered into a database, and blood and urine samples for genotyping and phenotyping have also been obtained. All of the assays to be performed have been refined in our laboratories.				
<b>14. Subject Terms (keywords previously assigned to proposal abstract or terms which apply to this award)</b> breast cancer, epidemiology				<b>15. NUMBER OF PAGES</b> 43
				<b>16. PRICE CODE</b>
<b>17. SECURITY CLASSIFICATION OF REPORT</b> Unclassified	<b>18. SECURITY CLASSIFICATION OF THIS PAGE</b> Unclassified	<b>19. SECURITY CLASSIFICATION OF ABSTRACT</b> Unclassified	<b>20. LIMITATION OF ABSTRACT</b> Unlimited	

20020124 190

## TABLE OF CONTENTS

<b>Front Cover</b>	1
<b>Report Documentation Page (SF 298)</b>	2
<b>Table of Contents</b>	3
<b>Introduction</b>	4
<b>Body</b>	5
<b>Key Research Accomplishments</b>	10
<b>Reportable Outcomes</b>	10
<b>Conclusions</b>	10
<b>References</b>	none
<b>Appendices</b>	12

## Introduction

Among African-American women younger than age 50, breast cancer incidence is almost twice that of Caucasian women. African-American women are more often diagnosed with aggressive tumors and have higher mortality rates than Caucasians. Differences in tumor biology and mortality do not appear to be due to factors related to socioeconomic status. Little is known regarding explanations for these racial disparities, perhaps because of the difficulty in enrolling African-Americans into research studies. The purpose of this pilot study was to develop a novel method of recruitment, focused primarily on minority women, and investigate previously unexplored risk factors in breast cancer epidemiology. Through rapid case ascertainment by tumor registries in Arkansas, we intend to enroll approximately 260 cases over two years, frequency-matched to controls randomly selected from Health Care Finance Administration (HCFA) and Arkansas Driver Services (ADS) lists. Cases and controls are matched to racially similar breast cancer survivor-recruiters. Potential participants are sent introductory postcards with the recruiters' photographs on them. Several days later, the recruiters call the potential participants to describe the study and seek their participation. Culturally appropriate interviewers administer questionnaires, draw blood and collect urine specimens from the participants. Once processed, data from these sources will be used to explore study hypotheses related to gene/environment interactions. We intend to evaluate the role that diet particular to African-Americans in the rural South may play in breast cancer etiology, and to assess the possible modification of risk by genetic differences in steroid hormone and carcinogen metabolism. A specimen bank was established to enable exploration of future hypotheses.

## Body

The proposed work was a pilot case-control study of breast cancer in African-American women. We realized, however, that without a comparable Caucasian group from the same locales as the African-American women, interpretation of the data would be difficult. It would be impossible to determine if specific risk factors are more prevalent in African-American women and are, thus, related to the increased early age at onset and more aggressive disease, or if they are merely regional habits that are shared by women of both groups. Therefore, additional funding was sought from the Public Health Service Office of Women's Health (DHHS PHS OWH) to support an identical study in Caucasian women, so that results could be compared. Although that study is conducted under a separate protocol, results will be discussed herein.

Research accomplishments associated with each Task outlined in the Statement of Work will be addressed within the context of each of the accomplishments.

### **Technical Objective 1 Develop and pilot a novel approach for enrolling minority women into research studies.**

#### **Task 1: Months 1-2: Organizational start up tasks--finalize questionnaire, continue training sessions and role-playing with Witness Project<sup>TM</sup> recruiters and interviewers.**

These tasks were accomplished in year 1. The questionnaire was finalized, interviewers are well-trained and experienced, and the recruiters are highly successful. Meetings are held regularly with recruiters to maintain enthusiasm and commitment, and to troubleshoot areas of difficulty. Presentations of recruitment strategies were made at two meetings (as well as the DOD meeting) in 2000: Keystone Conference in Taos entitled "*Molecular Epidemiology: A New Tool in Cancer Prevention*", and at the Annual Meeting of the American Association for Cancer Research.

#### **Task 2: Months 3-24. Identify incident breast cancer cases by rapid ascertainment; Identify controls from Department of Motor Vehicles and State Identity lists; recruitment of 230 cases and 230 controls by staff from Witness Project<sup>TM</sup>. Periodically assess effectiveness of individual recruiters by evaluation of response rates among women contacted by each individual.**

Recruitment of both cases and controls is ongoing. As reported last year, the study has not moved as quickly as anticipated, primarily because of the inability to identify sufficient numbers of African-American women with breast cancer who are eligible for the study. As reported in the previous annual reports, we expanded our case-ascertainment efforts to several other sites to increase numbers of African-American women who were eligible for our study. Although we had obtained IRB permission before making any of these changes to the protocol, we overlooked getting prior permission from the Department of Defense. We stopped case ascertainment and recruitment in May at the request of the USAMRMC until the changes are approved by DOD Human Subjects Protection. Because of difficulties in case ascertainment and

delays in the study, we have applied for a one-year no cost extension to continue the study until July 2002.

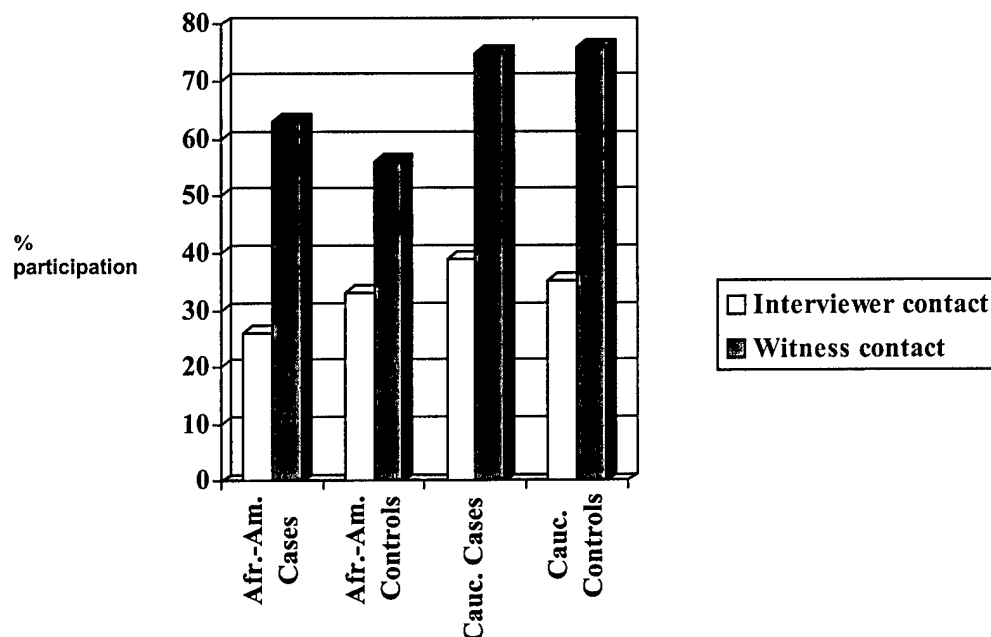
Another significant change is that the PI of the study, Dr. Christine B. Ambrosone, accepted a position to direct the Cancer Epidemiology Program at the Derald H. Ruttenberg Cancer Center, Mount Sinai School of Medicine, New York, New York, where she began in November, 2000. The funded study remains at NCTR and UAMS, and the same study personnel manage the day-to-day operations. Dr. Ambrosone is in constant communication with the study directors, and makes frequent return visits to Arkansas to monitor the progress of the study. During this period, Dr. Fred F. Kadlubar, Chair, Division of Molecular Epidemiology, NCTR, became the 'on-site' PI of the study. We have submitted all of these changes to the USAMRMC HSRRB and are awaiting approval from the UAMS IRB. Dr. Ambrosone will remain actively involved as the conceptual PI of the study, conferring frequently with Dr. Kadlubar.

Table 1 shows numbers of women enrolled into the study to date, and response rates for both African-American and Caucasian women. To date, interviews have been completed for 385 women, aged 29-75, 248 with breast cancer and 137 community controls. The participation rate (the proportion of women who complete the study) for cases is 76% for Caucasian women, and 61% for African-American women. These rates are much improved over those using the standard methodology employed in an earlier study in this difficult-to-reach community, in which, for Caucasians and African-Americans (men and women) combined, participation rates were 37% and 30% for cases and controls, respectively.

Table 1. Participation in Case-Control Study

African-American Women			
	Total contacted	Number Enrolled	Participation Rate
Cases	115	75	63%
Controls	95	30	56%
Caucasian Women			
	Total contacted	Number Enrolled	Participation Rate
Cases	361	270	75%
Controls	247	132	76%

**Task 3: Months 24-30 Calculate overall response rates for cases and controls, using Witness<sup>TM</sup> recruiters. Compare to those in UAMS earlier pilot study of breast cancer, among African-Americans in colon cancer study at UAMS, and in published epidemiologic studies in African-American populations.** As indicated in Table 1 and shown below, participation rates for African-American cases and controls are 63% and 56% respectively. In the earlier study of colon cancer at UAMS in this difficult-to-reach community, where potential participants were contacted by an interviewer in a standard method, participation rates were approximately 37% and 30% for cases and controls, for Euro-Americans and African-Americans (men and women) combined. Response rates will be examined in more depth at the completion of the study.



**Technical Objective 2** With a Food Frequency Questionnaire (FFQ) supplemented with foods commonly eaten by African-American women in the rural south, investigate the role of dietary sources of fat and heterocyclic amines in BC risk.

**Task 1: Months 1-3** Adapt FFQ to include foods found to be commonly eaten by African-American women in Eastern Arkansas previously surveyed.

As reported in the Annual Report for 1999, to determine if additional foods should be added to the Gladys Block Health Habits and History Questionnaire (HHHQ) to improve its suitability for African-American women in the lower Mississippi Delta, we conducted a survey of foods and cooking methods that may be particular to these residents. In collaboration with the Department of Dietetics and Nutrition at UAMS, a list of 60 foods commonly eaten by this population, such as wild game, parts of animals not traditionally eaten, and foods cooked with fat, was compiled through in-depth interviews and focus groups. We developed a Food Frequency Questionnaire with those foods elicited and then surveyed approximately 400 African-American women, aged 40 to 70, who live in eastern Arkansas. The survey indicated that few of the foods queried were eaten frequently by a large proportion of the population, but that several food items not on the Block questionnaire were eaten 1 to 4 times or more per month by > 50% of women surveyed. These foods included okra, southern peas (crowder, purple hull, split), butter and northern beans. Furthermore, more than 50% of women added fat when they cooked beans or greens, such as collards, mustard greens or kale. These additional items were added to the questionnaire already validated in Atlanta for a southern African-American population.

**Task 2: Months 3-26** Interviews with cases and controls; ongoing monitoring of interviewers.

As stated above, interviewing of cases and controls is ongoing. Completed questionnaire booklets are reviewed weekly by the project director, for accuracy and coherence. Interviewer performance is thus evaluated continually. One phenomenon that we have

observed using this methodology is the case in which a potential participant will agree when speaking with the recruiter, but then refuse when contacted by the interviewer. Late refusals may occur at the time the interview is scheduled, or after the interview is scheduled, often after several requests from the participant to reschedule. Interestingly, the pattern of late refusals varies by interviewer, and steps are being taken to train all interviewers in how to approach the potential participants who have already agreed to participate. We have also taken steps to cut down on the amount of time between the recruiter contact and the interview scheduling. Late refusal rates have dropped 20% since these changes were implemented.

**Task 3: Months 24-30 Double data entry, with ongoing quality control.**

Although this task was not targeted for until the beginning of year 3, we have developed a data base for entry of the questionnaire data. All data collected to date, have been double-entered and we are currently working on data checks and data cleaning for data that has been collected and entered. Interview data will be entered on an ongoing basis.

**Task 4: Months 30-36 Perform statistical data analysis; initial descriptive analyses, study of main effects of data derived from questionnaire.**

To be completed in the upcoming year.

**Technical Objective 3 Evaluate genetic variability in metabolism of HAs by examining phenotypic variability in CYP1A2 and sulfotransferase activity, as well as genetic polymorphisms in *NAT1* *NAT2*, *ST1A3* and *CYP1A2*.**

**Task 1: Months 3-26** Perform phenotyping assays for CYP1A2, NAT2, and phenol sulfotransferase.

These assays are ongoing, although analysis will not be evaluated by cases and controls until all data are collected. As reported last year, we did, however, use data for sulfotransferase, along with that from participants in a study of colorectal cancer, to evaluate correlations between data from the phenotyping and genotyping. The paper was published in Pharmacogenetics (see Appendix). DNA is extracted from specimens weekly, and genotyping will be performed beginning in the middle of the final year.



**Task 2: Months 26-30 Perform DNA analysis for genetic polymorphisms in *CYP1A2*, *NAT1*, *NAT2*, *ST1A3***

We established a biologic specimen bank in the context of this study, and protocols for processing and storage of blood were developed. Blood samples are processed so that there are aliquots of serum, plasma, platelets, red blood cells, and buffy coat. Using a processing system currently used in the 350,000-person EPIC study in Europe, each blood component is mechanically aliquotted into several 0.5ml straws that are prestamped with an ID number and barcode. Straws are heat-sealed and stored in canisters in liquid nitrogen tanks, with a detailed computerized mapping scheme in place. Our laboratory routinely performs high throughput genotyping and has extensive experience in assaying all of the genes proposed for study. Because we will extend the study for another year due to slow case ascertainment, genotyping will begin in this final year of the study.

**Task 3: Months 31-36 Merge data from laboratory results with questionnaire data base. Perform statistical analysis for main effects of polymorphisms evaluated by phenotyping and genotyping. Evaluate interactive effects of laboratory data and questionnaire data.**

To be completed in the upcoming year.

## Key Research Accomplishments

- Establishment of infrastructure for molecular epidemiologic study (questionnaire development, protocols and equipment for blood processing and specimen banking, recruiter and interviewer hiring and training, development of data bases for participant tracking and questionnaire data, etc.), data entry.
- Enrollment of cases and controls into study – response rates far superior to those in earlier case-control study in the same locales.
- Adaptation of FFQ to the African-American population in Arkansas.

## Reportable Outcomes

### Publications:

Nowell S, Ambrosone CB, MacLeod SL, Mrackova G, Williams S, Plaxco J, Ozawa S, Kadlubar FF, Lang NP. Relationship of phenol sulfotransferase (SULT1A1) genotype to sulfotransferase activity in platelet cytosol *Pharmacogenetics* 2000;10:789-797.

Moss RA, Erwin DO, Morris-Chatta R, Long S, Ambrosone CB. Challenges, limitations and strategies for increasing participation in epidemiologic studies: a novel approach to recruiting African-Americans. *Annals of Epidemiology* (submitted). A similar paper was submitted in 1999 to two journals, but was rejected due to 'low priority'. The paper has been substantially rewritten, with more complete data available now, and was submitted to *Annals of Epidemiology*.

**Biologic specimen bank** established with DNA, serum, plasma and red blood cells from cases and controls.

### Grant funded based upon recruitment methodology and pilot data:

"Genetic factors in breast cancer: Center for interdisciplinary biobehavioral research" (Center Grant 07/01/2001–06/30/2005, Bovbjerg, PI)

"Behavior, estrogen metabolism and breast cancer risk: a molecular epidemiologic study", Ambrosone (PI)

## Conclusions

In this pilot study, case ascertainment has been accomplished through collaborations with physicians at the Arkansas Cancer Research Center (ACRC) in Little Rock; Jefferson Regional Medical Center (JPMC) in Pine Bluff, Arkansas; Methodist Healthcare Cancer Center (MHCC) in Memphis, Tennessee and through the Arkansas State Department of Health. Most recently, we have received the cooperation of three additional breast surgeons, practicing at 3 different large hospitals in Little Rock and access to Tumor Registries of two of those hospitals. Controls have been identified through the Health

Care Finance Administration and Arkansas Driver Services enrollment files. Interviews have been conducted and data double-entered into a database. Blood and urine samples for genotyping and phenotyping have also been obtained. We have established a biologic specimen bank, with a detailed protocol for blood processing and storage. All of the assays to be performed have been refined in our laboratories at the ACRC and National Center for Toxicological Research (NCTR). Recruitment is well underway and the methodology appears to be a vast improvement over previous work in this area.

Full-scale epidemiologic studies require large budgets, which include personnel, supplies, equipment, etc. Furthermore, building of an infrastructure is essential, yet laborious and time-intensive. There was little to no organized epidemiologic studies being conducted in Arkansas when this study was initiated, and funding received from the Department of Defense and the OWH has been used to develop the infrastructure and get it the study into the field. We now have cooperation from several major and physicians and expect to more quickly ascertain African-American women diagnosed with breast cancer in the coming year. Our methodology has been established and tested, staff training manuals have been developed and successfully piloted, and a specimen bank has been established and is in use. Data from this pilot study was used to support a successful grant application to conduct a similar study among African-American women in New York city.

**Appendix:**

Published manuscript

Submitted manuscript

Challenges, Limitations and Strategies for Increasing Participation in Epidemiologic  
Studies: A Novel Approach to Recruiting African-Americans

R.A. Moss, D.O. Erwin<sup>1</sup>, R. Morris-Chatta<sup>1</sup>, S. Long<sup>1</sup>, C.B. Ambrosone<sup>1,2 \*</sup>

<sup>1</sup>Arkansas Cancer Research Center, University of Arkansas for Medical Sciences, Little  
Rock, AR 72205

<sup>2</sup>National Center for Toxicological Research, Division of Molecular Epidemiology,  
Jefferson, AR 72079

\*Address correspondence to:

Christine B. Ambrosone, Ph.D.

Derald H. Ruttenberg Cancer Center

Mount Sinai School of Medicine Box 1150

One Gustave L. Levy Place

New York, NY 10029

Email: [christine.ambrosone@mssm.edu](mailto:christine.ambrosone@mssm.edu)

Tel: 212 659-5552

Fax: 212 849-2564

Running title: Participation in epidemiologic studies

## ABSTRACT

Purpose: Among African-American women younger than age 50, breast cancer incidence is almost twice that of European-American women. African-American women are more often diagnosed with aggressive tumors and have higher mortality rates than European-Americans. The reasons for the disparities have not been completely elucidated, and there are few epidemiologic studies addressing breast cancer specifically in African Americans. The purpose of this paper is to introduce a novel approach used to recruit African-American women to an epidemiologic study. This method may prove useful in recruiting for similar studies and for clinical trials.

Methods: Building upon a strategy in which African-American women who survived breast cancer were recruited, trained, and promoted as role models and lay health advisors (Witness Project®) in the medically-underserved Mississippi Delta region, we describe a novel methodology developed for recruitment of African-American women. This model is being used in an ongoing molecular epidemiology study of risk factors for breast cancer.

Conclusions: Although the study is ongoing, this survivor-based method of recruitment of African-Americans addresses many of the barriers to participation in research studies, and the recruitment methodology may be useful to other epidemiological studies in this population.

## INTRODUCTION

### *Breast Cancer in African-American Women*

Among African-American women younger than age 50, breast cancer incidence is almost twice that of European-American women. African-American women are more often diagnosed with aggressive tumors and have higher mortality rates than European-American (1). While Caucasian women with breast cancer have a 5-year survival rate of 79%, only 62% of African-American women remain living 5 years after diagnosis (2). While little progress has been made in identification of modifiable risk factors for breast cancer, the more aggressive tumor biology and higher mortality in African-American women, and the higher incidence in younger women is particularly troubling. Factors contributing to increased breast cancer incidence among younger African-American women are unknown, and differences in survival do not appear to be related solely to socioeconomic status (2,3). Little is known regarding explanations for these ethnic disparities, and these gaps in knowledge may be due, in part, to the difficulty of enrolling African-Americans into research studies. In general, poor participation of potential cases and controls is becoming a growing problem in all research studies, but it is of particular concern for studies of minority health.

There is a large African-American population in the Mississippi Delta region of Eastern Arkansas, the majority of whom live in rural, low-income areas. As shown in Table 1, data from the Centers for Disease Control data demonstrate that breast cancer mortality rates for both Euro-American and African-American women in this region are higher than the national average, and this is most notable among African-American women. In an earlier

case-control study of cancer in central and eastern Arkansas, recruitment of subjects into the study was extremely difficult and participation rates were low. Potential participants were contacted by telephone, and response rates were approximately 30% for controls, and 37% for cases.

We describe an interdisciplinary pilot study to evaluate the effectiveness of a novel method of recruitment in this population. The study was designed to develop an infrastructure and methodology for conducting breast cancer research in the Delta region, and to explore hypotheses regarding genetic and environmental factors that could explain ethnic disparities in patterns of breast cancer incidence and pathology.

#### *Non-Participation of Minorities in Research Studies*

Poor participation in epidemiologic studies is becoming an increasingly significant problem in research. Those who do consent may not be representative of the general population, particularly controls, who are the least motivated to participate in research. This selection bias and poor overall participation may result in observation of spurious associations, or an inability to detect true risk associations. Recruitment of African-Americans to research studies is historically difficult (4), and response rates by race are often not detailed in study reports. Barriers to minority recruitment have been attributed to sociocultural, economic and individual factors (4). Much of the groundwork for the study of barriers to participation and generation of strategies to increase participation in biomedical studies has been laid in the field of clinical trials. Ness and colleagues (5) reviewed the literature in 1997 and concluded that "the published literature currently contains insubstantial data to either refute



or prove that there are differential recruitment rates among minorities as compared with whites” in clinical studies. This review of published literature between 1993 and 1995, however, reflected research generally accomplished several years prior to the 1993 Revitalization Act in which NIH stated that minority groups must be included as subjects in clinical research; thus, reporting of recruitment difficulties may not have been relevant. Other national data in this genre suggest that minorities may be underrepresented in clinical trials (4,6,7). Lack of participation and accrual has been attributed to a variety of barriers. These include: treatment cost problems due to low socioeconomic status, lack of bilingual or culturally sensitive staff, perceived efficacy of investigational programs or trials, lack of protocol availability for minorities related to lower eligibility/late diagnosis, lack of community involvement and support, and difficulties related to poverty, such as hopelessness, powerlessness, and survival priorities (4,5,8-14).

Those sub-populations unfamiliar with the nature of research studies or those who are wary of the medical community may be most difficult to recruit. For example, the recruitment of African-Americans into research studies is historically difficult (4) perhaps because of the extensive knowledge among African-Americans of the Tuskegee Syphilis Experiment. That breach of ethics in a research study may have created a sense of “distrust and suspicion that hampers cancer research efforts in many Black American communities” (13,14). In a comprehensive review of the literature on recruitment for controlled clinical trials, Lovato (7) and colleagues list “lack of trust” as one of the most common barriers to participation. Because barriers to recruitment may vary among age and ethnicity subgroups, recruitment strategies need to be tailored to specific potential participants (15,16). El- Sadr

and Capps (6) have suggested that attention should be given to characteristics of the investigator to reduce the suspicion and distrust that may discourage participation in research studies.

In a review of the literature, Kelly and Cordell (17) reported that recruitment and retention of women is significantly improved (enrolled at twice the rate) in studies in which some or all of the principal investigators are female as compared to studies in which all of the investigators were male. While gender of the investigator has been shown to be important, cultural background may be even more so. Interestingly, although interviewer effects have been considered less important in telephone surveys, Moorman and colleagues (15) found in the North Carolina Breast Study that results of even the first contact by phone were dependent on or influenced by the perceived ethnicity of the interviewer. While cooperation rates were highest among both black and non-black women when interviewers and participants were concordant on ethnicity in this study, cooperation rates were lowest among controls, older women, and African-American women with breast cancer in comparison to Euro-American women. For African-Americans, the use of African-American interviewers may be reassuring and better demonstrate that the research is important and relevant to people of their community, thus increasing the likelihood of overcoming a mistrust of medical researchers (6,15). The ability of the nine Minority-Based Clinical Community Oncology Programs (MB-CCOP) to accrue 10% of all minority participants to NCI-sponsored oncology trials using minority recruiters further supports the importance of cultural concordance in the staff (18).

The recruitment of minority participants to research studies often requires more time and money than accrual of Caucasians (19), with the use of additional targeting strategies. In light of the intensity of the time and effort required, El- Sadr and Capps (6) have even suggested that the definition of research costs should be broadened to include the funding of meals, social and outreach services, transportation, child care, and educational materials to enhance the success of minority recruitment. Moreover, they stress the importance of a "realistic estimation of the number and types of personnel needed to successfully recruit, retain, and follow" trial participants.

Swanson and Ward (4) conclude that the most effective method of recruitment is from the community rather than the health care system. Likewise, Lovato et al. (7) listed successful recruitment strategies in minority populations as those programs that honor important cultural values (12) and utilize community leaders and relationships with community gatekeepers, including one-to-one communication strategies (20,21). In an intensive effort to enroll minorities in the Prostate Cancer Prevention Trial, Moinpour et. al defined the following issues hindering minority accrual: the need for a long-term infrastructure within the community; health interventions prior to recruitment efforts to help build trust; long-term presence of the minority recruiter in the community; including the minority recruiter on the site staff team (22). Freimuth reports that in the African-American population, there is a "preference for ordinary African-American people who had experienced and overcome a problem" (23). Drawing upon this observation, and to specifically address the sociocultural, economic and individual factors that have been identified as barriers to minority recruitment, the Witness Project® is an ideal model for recruiting participants to

epidemiologic studies. The Witness Project® is an outreach program in which rural and lower income African-American women who have had early stage breast cancer share their experiences in church and community groups to encourage and educate other women about early detection (24,25).

#### *Description of the Witness Project*

The Witness Project® was developed to increase awareness of breast and cervical cancer and to encourage screening for early detection in rural African-American communities. In this program, African-American women who are breast and cervical cancer survivors are recruited, trained, and promoted as role models and lay health advisors. These women speak at rural churches and in community settings about the need for breast self-examination, clinical breast exams, screening mammography, and Pap tests (24). Begun in 1991, the Witness Project® has effectively increased breast cancer awareness and screening behaviors among African-American women. Witness® volunteers also participate as Arkansas Cancer Research Center co-facilitators in psychological interventions, and provide a support network for other women diagnosed with breast cancer. Results from the Witness Project® indicate that this program is an effective method to reach and communicate with minority women (26). Furthermore, post-intervention surveys of participants demonstrate a significant increase in the practice of breast self-examination and mammography as compared to women in the control group.

Although the Witness Project® was designed for reaching rural African-American women for the purpose of increasing early detection, it is postulated that this role model method will be effective for recruitment of women to epidemiological studies. This type of initiative is

intended to address the social and cultural issues of credibility and trust that have been identified as barriers to research. It is proposed that survivors as role models and messengers are better able to educate and inform their peers in a culturally appropriate way because they share spiritual and cultural lifestyles and beliefs that increase the level of trust in the relationship.

The *Patient Advocates for Clinical Trials (PACT)* project is patterned after the Witness Project®. This *PACT* project was a two-year study designed to investigate the feasibility of overcoming sociocultural barriers to participation in clinical research through systematic use of breast cancer survivors serving as role models to inform other women about clinical trials. Nineteen women who had participated in a breast cancer clinical trial were trained as *PACT* advisors, and these women also served as models and contributors to methodology for recruitment to epidemiologic studies.

### *Study Design*

The present study is designed to investigate breast cancer in both African-American and European-American women in the Delta Region, evaluating diet specific to this population, and possible modification of risk by metabolic variability. Recruiters were drawn from the Witness Project® and the *PACT* project, and the recruitment was modeled on the successes of these two programs. Prior to initiating the project, ethics review was conducted by the University of Arkansas for Medical Science. Approval was given for all procedures, letters and informational material to be used in the study.

### Focus groups and training:

Through focus groups conducted among women who were Witness role models, we determined that it was important for only women to be interviewers for the breast cancer study. These groups helped to develop a script for recruiters, informational material for subjects, as well as a training manual for recruiters and interviewers. Relying on data obtained from focus groups and interviews, training procedures were standardized in a training manual. Recruiters are drawn from the Witness role models as well as the PACT advisors, all breast cancer survivors. During training sessions, recruiters participate in role-playing in order to consider the numerous possible reactions potential participants may have when contacted and to learn how to respond to each. By the end of training, recruiters understand the importance of having good response rates for valid results.

Case and Control Ascertainment: Cases diagnosed throughout Arkansas are ascertained in cooperation with pathology departments and tumor registries at several hospitals. Using the rapid case ascertainment system, all African-American women between the ages of 20 and 75 residing in the 75 Arkansas counties who have newly diagnosed primary, incident, histologically-confirmed invasive breast cancer are eligible for study inclusion and are recruited within three to six months of diagnosis. Controls are selected from the State of Arkansas Driver's license and State identity card list, and from Health Care Finance lists, and are frequency-matched to cases on age and county of residence. Recruiters are matched by ethnicity and urban or rural residency to potential participants in the study. After cases are identified and notified by their doctors of the study, and controls are sent letters from Driver Services and HCFA, Witness recruiters are matched to them by town or county of residence. A postcard is sent to the potential participant by the matched recruiter,

with a photo of the recruiter on it (Figure 1). The card introduces the recruiter and describes her reasons for contacting the recipient in the near future. Several days later, the recruiter makes a telephone call to describe study goals and to discuss the interview procedure, following a basic script that was tested in focus groups and role-played with the Witness recruiters. If the woman agrees to participate, her name is given to an African-American interviewer, who phones to make an appointment for the interview and signed consent process, either at the participant's home or another agreed-upon location, such as the woman's hospital or clinic. Witness staff maintains contact with patients throughout the study to provide assistance as necessary.

Data collection: Interviewers of similar racial background conduct in-person interviews with cases and controls. If the individual agrees to participate, an interviewer of the same ethnicity schedules an interview, either at the participant's home or at another convenient location. Informed consent is obtained, a blood and urine specimen collected and the interview administered. The interview questionnaire is designed to ascertain dietary habits, hormonal factors, and other potential breast cancer risk factors.

Remuneration: Recruiters are paid \$10 for successful contacts and \$5 for those who refuse. Participants in the study are offered a \$25 gift certificate to one of several local and national retailers. Many people in the Delta live in extreme poverty, and in our experience, this small remuneration not only increases incentive to participate, but is also greatly appreciated.

### Preliminary Analysis of Participation

Although this study was not designed to test recruitment methodologies, there are noted improvements in response rates from previous work in this region. Breast cancer survivors are enthusiastic about serving as part of a "recruitment team" and 63% of the new African-American patients deemed eligible for the study were subsequently enrolled after talking with a recruiter. To date, interviews have been completed for 75 African-American women, aged 29-75, with breast cancer. Enrollment of community controls is in the active recruitment phase, with 95 contacted and 56% enrolled. Preliminary participation rates for European-American women are 75% for cases, and 76% for controls. While not designed as a formal comparison, it is estimated that these rates are much improved over those using the standard methodology employed in an earlier study in this difficult-to-reach community, in which participation rates were approximately 37% and 30% for cases and controls, respectively for Euro-Americans and African-Americans (men and women) combined (Figure 2).

### Modification of methods

We actively monitor and evaluate recruitment efforts to improve participation rates. To maintain enthusiasm and motivation of the staff, recruiters are paired with an interviewer 'buddy' who contacts them weekly. These staff members build rapport, provide a venue for open communication, and encourage feedback. This feedback is often implemented in study methodology improvements. The recruiters have a unique perspective on the recruitment process, as cancer survivors and key informants. Quarterly 'barbecues' are held as opportunities to meet on a social and professional level. The quarterly meetings include discussion of necessary changes in the protocol, sharing success stories, and watching



videotapes of each recruiter making telephone contacts. Successful strategies are incorporated into recruiters' scripts, and the tapes are used in training new recruiters.

Recruiter satisfaction has increased as a result of additional focus on the recruitment role, reducing turnover. Recruiters have become active participants in the process of adjusting study methods to this population. For example, when they discovered that older women were particularly reluctant to give a blood sample, a new protocol was introduced to perform the necessary studies on a sample of buccal mucosa. Experience appears to play a large role in recruitment success; it is hoped that increased satisfaction will lead to long tenure in the recruiter role.

One phenomenon that we have observed using this methodology is the case in which a potential participant will agree when speaking with the recruiter, but then refuse when contacted by the interviewer. Late refusals may occur at the time the interview is scheduled, or after the interview is scheduled, often after several requests from the participant to reschedule. Interestingly, the pattern of late refusals varies by interviewer, and steps are being taken to train all interviewers in how to approach the potential participants who have already agreed to participate based on feedback from recruiters in a process of continual adjustment. We have also taken steps to reduce the amount of time between the recruiter contact and the interview scheduling.

### *Discussion*

Using the patient advocate model developed in the *Witness Project*® and the *PACT* project, we developed an innovative method of recruitment that has shown initial success. While the study is not designed to be directly compared to previous attempts to accrue African-American participants in epidemiologic studies, using patient advocates is designed to specifically address previously identified barriers to participation in clinical trials by African-Americans such as health beliefs, social relationships, and lack of trust of medical authorities.

This model addresses the issue of retention of minorities to clinical trials by establishing a social network within the formal ongoing training program with events such as the quarterly barbecue. This is the kind of event El-Sadr and Capps (6) found useful in the recruitment and retention of minority participants, and was accordingly included in the budget.

It also directly addresses the issues hindering minority accrual defined by Moinpour et. al (22), specifically the need for a long-term infrastructure within the community; health interverentions prior to recruitment efforts to help build trust; long-term presence of the minority recruiter in the community; including the minority recruiter on the site staff team. The recruitment process can also serve as a source of support and networking for women newly diagnosed with breast cancer, and a source of positive affirmation for the patient advocates who work as recruiters.

### *Conclusion*

To understand the causes of more aggressive tumors and an earlier age of onset of breast cancer among African-American women, it is essential to devise strategies to encourage minority participation in research studies. Using patient advocates as minority recruiters is a novel method of recruitment of African-Americans to epidemiological as well as other studies. The study is ongoing, and the model described here for this epidemiological study of breast cancer is also currently being used in a case-control study of prostate cancer.

Although further evaluation of this method is planned, these preliminary process evaluations indicate that this is a promising strategy for optimizing the recruitment of epidemiological study participants. In addition, it is an important collaborative step in increasing the role and contribution of survivors and advocates into the research design process.

## **ACKNOWLEDGMENTS**

The authors wish to acknowledge the following individuals, who through their devotion to their communities as cancer survivors and dedication to preventing breast and prostate cancer, have enhanced our ability to conduct this research: Laurita Irvin, Linda Creggett, Joanne Helgeson, Lillie Watson, Christine Oliver, Gwendolyn Hill, John Youngblood, Cleovis Whiteside, Artis Mendenhall, and Max Terrell, Ph.D. In addition, we are grateful to the following research interviewers: Cecelia Twillie-Woods, Rachel Butler-Green, Bobbi Lawson, Kristin Bondurant, Sean Walls and Shelley Sontag and to Shirley Gray for editorial assistance.

This work was supported, in part, by grants from the Department of Defense Breast Cancer Research Program (DAMD17-98-I-A800) and the Food and Drug Administrations's Office of Womens' Health.

## REFERENCES

1. Marbella AM, Layde PM. Racial trends in age-specific breast cancer mortality rates in US women. *Am J Pub Health* 2001;91:118-21.
2. Miller BA, Feuer EJ, Hankey BF. Recent incidence trends for breast cancer in women and the relevance of early detection: an update [see comments]. *Ca: a Cancer Journal for Clinicians* 1993;43:27-41.
3. Jones BA, Kasl SV, Curnen MG, Owens PH, Dubrow R. Can mammography screening explain the race difference in stage at diagnosis of breast cancer? *Cancer* 1995;75:2103-13.
4. Swanson GE, Ward AJ. Recruiting minorities into clinical trials: toward a participant-friendly system. *J Natl Cancer Inst* 1995;87:1747-59.
5. Ness RB, Nelson DB, Kumanyika SK, Grisso JA. Evaluating minority recruitment into clinical studies: how good are the data? *Ann Epidemiol* 1997;7:472-78.
6. El-Sadr W, Capps L. The challenge of minority recruitment in clinical trials for AIDS. *JAMA* 1992;267:954-57.

7. Lovato LC, Hill MJ, Hertzmark E, Hunter CF, Probstfield JL. Recruitment for controlled clinical trials: Literature summary and annotated bibliography. *Controlled Clinical Trials* 1997;18:328-57.
8. Freeman, H. P. The impact of clinical trial protocols on patient care systems in a large city hospital. *Cancer* 72(4 Suppl), 2834-2838. 1993.
9. Brawley OW, Tejeda H. Minority inclusion in clinical trials issues and potential strategies. *Journal of the National Cancer Institute Monographs* 1995;17:55-57.
10. Modiano, M., Vilar-Werstler, P., Lash, S., and et.al. Increasing enrollment of minorities into clinical trials through community involvement. *Proc Annu Meet Am Soc Clin Oncol* 11, A409. 1992.
11. Million-Underwood S, Sandler E, Davis M. Determinants of participation in state-of-the-art cancer prevention, early detection/screening, and treatment trials among African-Americans. *Cancer Nursing* 1993;16:25-33.
12. Roberson N. Clinical trial participation: viewpoints from racial/ethnic groups. *Cancer* 1994;74:2687-91.
13. Harris Y, Gorelick PB, Samuels P, Bempong I. Why African Americans may not be participating in clinical trials. *J Natl.Med.Assoc* 1996;88:630-34.
14. Simon MS, Brown DR, Du W, LoRusso P, Kellogg CM. Accrual to breast cancer clinical trials at a university-affiliated hospital in metropolitan Detroit. *Am J Clin Oncol* 1999;22:42-46.

15. Moorman PG, Newman B, Millikan R, Tse C-KJ, Sandler DP. Participation rates in a case-control study: the impact of age, race and race of interviewer. *Ann Epidemiol* 1999;9:188-95.
16. Grunbaum JA, Labarthe DR, Ayars C, Harris R, Nichaman MZ. Recruitment and enrollment for project heartbeat: achieving the goals of minority inclusion. *Ethnicity Dis* 1996;6:203-12.
17. Kelly PJ, Cordell JR. Recruitment of women into research studies: a nursing perspective. *Clin Pharmacol Ther* 1996;10:25-28.
18. Hsing AW, McLaughlin JK, Hrubec Z, Blot WJ, Fraumeni JF, Jr. Tobacco use and prostate cancer: 26-year follow-up of US veterans. *Am J Epidemiol* 1991;133:437-41.
19. Lewis CE, George V, Fouad M, Porter V, Bowen D, Urban N. Recruitment strategies in the Women's Health Trial: Feasibility study in minority populations. *Controlled Clinical Trials* 1998;19:461-76.
20. Baines CJ. Impediments to recruitment in the Canadian national breast screening study: response and resolution. *Controlled Clinical Trials* 1984;5:124-40.
21. Kaluzny A, Brawley OW, Garson-Angert D, Shaw J, Godley P, Warnecke R *et al.* Assuring access to state-of-the-art care for U.S. minority populations: the first two years of the minority-based community clinical oncology program. *J Natl Cancer Inst* 1993;85:1945-50.

22. Moinpour CM, Atkinson JO, Thomas SM, Underwood SM, Harvey C, Parzuchowski J *et al.* Minority recruitment in the prostate cancer prevention trial. *Ann Epidemiol* 2000;10:S85-S91.
23. Freimuth VS. Narrowing the cancer knowledge gap between whites and African-Americans. *Monographs - Natl Cancer Inst* 1993;14:81-91.
24. Erwin DO, Spatz TS, Turturro CL. Development of an African-American role model intervention to increase breast self-examination and mammography. *J Cancer Educ*. 1992;7:311-19.
25. Bailey EJ, Erwin DO, Belin P. Using cultural beliefs and patterns to improve mammography utilization among African-American women: the Witness Project. *J Natl.Med.Assoc* 2000.Mar.;92.(3):136.-42. 2000;92:136-42.
26. Erwin DO, Spatz TS, Stotts RC, Hollenberg JA. Increasing mammography practice by African American women. *Cancer Practice* 1999;7:78-85.

Table 1. Age-adjusted breast cancer mortality rates per 100,000 in 1995. CDC, 1998

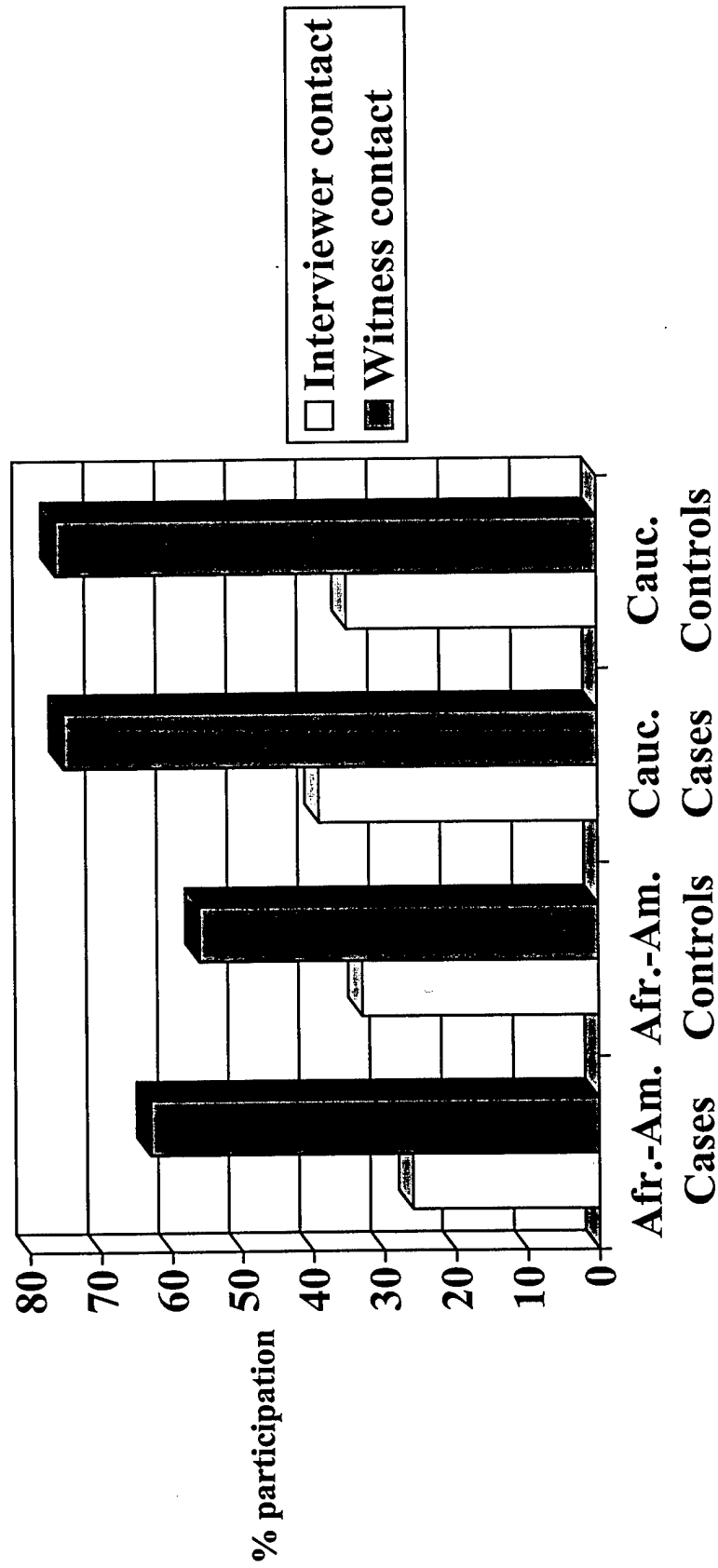
	African-American	Caucasian
Arkansas	35.7	21.4
Louisiana	31.7	26.5
Tennessee	31.4	24.3
Average US rates	27.4	22.7



## Figure Legends

Figure 1. Postcard sent to potential 'control' participant, matched with recruiter by age, race and county of residence.

Figure 2. Participation Rates: Usual Method vs. Witness® Volunteer Method



## Relationship of phenol sulfotransferase activity (*SULT1A1*) genotype to sulfotransferase phenotype in platelet cytosol

Susan Nowell<sup>a</sup>, Christine B. Ambrosone<sup>a,b</sup>, Shogo Ozawa<sup>d</sup>, Stewart L. MacLeod<sup>a</sup>, Gabriella Mrackova<sup>b</sup>, Suzanne Williams<sup>a</sup>, Jason Plaxco<sup>a</sup>, Fred F. Kadlubar<sup>b</sup> and Nicholas P. Lang<sup>a,c</sup>

<sup>a</sup>University of Arkansas for Medical Sciences, Surgical Oncology Department, Little Rock, AR, <sup>b</sup>Division of Molecular Epidemiology, National Center for Toxicological Research, Jefferson, AR, <sup>c</sup>Central Arkansas Veteran's Health Care System, Little Rock, AR, USA and <sup>d</sup>Division of Pharmacology, National Institute of Health Sciences, Tokyo, Japan

Received 23 November 1999; accepted 24 June 2000

Sulfation catalysed by human cytosolic sulfotransferases is generally considered to be a detoxification mechanism. Recently, it has been demonstrated that sulfation of heterocyclic aromatic amines by human phenol sulfotransferase (*SULT1A1*) can result in a DNA binding species. Therefore, sulfation capacity has the potential to influence chemical carcinogenesis in humans. To date, one genetic polymorphism (Arg<sup>213</sup>His) has been identified that is associated with reduced platelet sulfotransferase activity. In this study, data on age, race, gender, *SULT1A1* genotype and platelet *SULT1A1* activity were available for 279 individuals. A simple colorimetric phenotyping assay, in conjunction with genotyping, was employed to demonstrate a significant correlation ( $r = 0.23$ ,  $P < 0.01$ ) of *SULT1A1* genotype and platelet sulfotransferase activity towards 2-naphthol, a marker substrate for this enzyme. There was also a difference in mean sulfotransferase activity based on gender (1.28 nmol/min/mg, females; 0.94 nmol/min/mg, males,  $P = 0.001$ ). DNA binding studies using recombinant *SULT1A1*\*1 and *SULT1A1*\*2 revealed that *SULT1A1*\*1 catalysed *N*-hydroxy-aminobiphenyl (*N*-OH-ABP) DNA adduct formation with substantially greater efficiency (5.4 versus 0.4 pmol bound/mg DNA/20 min) than the *SULT1A1*\*2 variant. A similar pattern was observed with 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5b]pyridine (*N*-OH-PhIP) (4.6 versus 1.8 pmol bound/mg DNA/20 min). Pharmacogenetics 10:789–797 © 2000 Lippincott Williams & Wilkins

**Keywords:** Sulfotransferase, interindividual variability, phenotype, genotype, chemical carcinogenesis.

### Introduction

Human cytosolic sulfotransferases (SULTs) catalyse the transfer of the sulfonyl moiety from 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to the hydroxyl, sulfhydryl, amino or *N*-oxide groups of a variety of endo- and xenobiotics (Jakoby & Ziegler, 1990). This reaction generally results in more polar metabolites, thus facilitating their elimination from the organism. At least eight SULT isoforms have been identified in humans (Wood *et al.*, 1994; Her *et al.*, 1995, 1996; Rastogi *et al.*, 1996; Wood *et al.*, 1996; Her *et al.*, 1997; Weinshilboum *et al.*, 1997; Her *et al.*, 1998;

Sakakibara *et al.*, 1998) and are divided into two subfamilies, the phenol SULTs and the hydroxysteroid SULTs. SULT enzymes have a widespread tissue distribution and are expressed in the liver, lung, brain, skin, breast, kidney, gastrointestinal tissue and, of significance to molecular epidemiologic studies, in blood platelets (Hart *et al.*, 1979; Cappiello *et al.*, 1990; Zou *et al.*, 1990; Kudlacek *et al.*, 1995; Falany & Falany, 1996a,b; Hume *et al.*, 1996). Platelets express *SULT1A1* ('thermostable' sulfotransferase) and *SULT1A3* ('thermolabile' sulfotransferase) (Anderson *et al.*, 1998; Frame *et al.*, 2000), and these activities have been demonstrated in other blood cell types (Anderson *et al.*, 1991). In the case of *SULT1A1*, the activity found in platelets correlates with that found in other tissues (Young *et al.*, 1985; Coughtrie, 1996) and is thought to be coordinately regulated. Platelet SULT activity has a strong genetic

Correspondence to Susan Nowell, University of Arkansas for Medical Sciences, VA Research Slot 151, 4300 West Seventh Street, Little Rock, AR 72205, USA  
Tel: +1 501 257 4808; fax: +1 501 257 4822; e-mail: nowellsusana@exchange.uams.edu

component, as evidenced by the substantial interindividual variability in the expression of this enzyme and its heritability, which has been estimated between 0.83 and 0.96 (Reveley *et al.*, 1982).

Although traditionally considered Phase II detoxification enzymes, SULTs have also been implicated in the bioactivation of dietary and environmental procarcinogens (Miller, 1994; Glatt, 1997). Following *N*-oxidation by hepatic cytochrome P450, sulfation of a variety of *N*-hydroxylated arylamines and arylamides produces reactive esterified metabolites (Abu-Zeid *et al.*, 1992; Gilissen *et al.*, 1994). We have shown that several *N*-hydroxy metabolites of arylamine and heterocyclic amine carcinogens, including 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5b]pyridine (*N*-OH-PhIP) and *N*-hydroxy-aminobiphenyl (*N*-OH-ABP), can be activated to DNA binding species via sulfation by SULT1A1 (Ozawa *et al.*, 1994; Chou *et al.*, 1995a,b). Exposure to dietary and environmental procarcinogens has been strongly linked to the development of urinary bladder and gastrointestinal cancer (Massaad *et al.*, 1992; Gonzalez, 1995; Anderson *et al.*, 1997; Windmill *et al.*, 1997). Therefore, genetic and phenotypic factors involved in interindividual variability of SULT1A1 activity potentially play a key role in individual susceptibility to disease.

Recently, genetic polymorphisms have been identified in *SULT1A1* (Raftogianis *et al.*, 1997; Ozawa *et al.*, 1998). In particular, one *SULT1A1* polymorphism results in an amino acid change (Arg to His, designated *SULT1A1*\*1 and *SULT1A1*\*2) at the conserved residue 213. In a predominantly Caucasian population, the allele frequency was 0.674 and 0.313 for *SULT1A1*\*1 and *SULT1A1*\*2, respectively. The *SULT1A1*\*2 allele was associated with reduced sulfotransferase activity and thermostability in platelets, although the relationship to activity in human liver cytosol was not clear (Raftogianis *et al.*, 1997; Ozawa *et al.*, 1998). Coughtrie *et al.* (1999) investigated the frequency of this allele in both a Caucasian and Nigerian population and found no significant differences. This is in contrast to other drug metabolizing enzymes where there is considerable ethnic variation (Critchley *et al.*, 1986; Kalow, 1991; Stephens *et al.*, 1994; Llerena *et al.*, 1996; Beutler *et al.*, 1998; Walker *et al.*, 1998). In this study, we employed genotyping along with a simple colorimetric activity assay to evaluate the degree to which the *SULT1A1* genetic polymorphism correlates with platelet SULT activity towards 2-naphthol. Since the assay is designed to reflect the catalytic activity of only SULT1A1 in platelets, these findings demonstrate the utility of genotype analysis as a contributor to SULT1A1 phenotype.

## Materials and methods

### Study participants

These data were derived from ongoing case-control studies designed to investigate the role of genotypic and phenotypic variability in modification of risk of colorectal and breast cancer associated with dietary heterocyclic amines and steroid hormones. Participants included those diagnosed with incident, primary, histologically confirmed cancer of the colon/rectum or breast, as well as community control subjects. Control subjects were frequency matched to cases by race, age and county of residence. Exclusion criteria for the case-control study included a history of cancer (other than non-melanoma skin cancer), uncontrolled cardiovascular disease, hepatic dysfunction as determined by bilirubin > 1.5 mg/dl, SGOT > 40 U/l, alkaline phosphatase > 140 U/l, and abnormal renal function as determined by BUN > 20 mg/dl and serum creatinine > 1.8 mg/dl. After obtaining informed consent from each participant according to an institutional review board approved consent protocol, a 24 ml blood sample was collected in four Vacutainer tubes (Becton Dickinson, Fisher Scientific, Houston, TX, USA) containing ascorbate citrate dextrose to prevent platelet aggregation. Blood samples were stored at room temperature until processed and platelets were isolated from other cell types within 24 h of collection. Genotyping and phenotyping data were available for 279 participants.

### Materials

Histopaque 1119 and 1077, 4-nitrophenyl sulfate, PAPS, calf thymus DNA (type I) and 2-naphthol were obtained from Sigma Chemical Company (St Louis, MO, USA). Other reagents used in the formulation of buffers were purchased from Sigma and were of the highest quality available. *Hae*II was purchased from New England Biolabs (Beverly, MA, USA). Taq DNA polymerase, along with other polymerase chain reaction (PCR) reagents was purchased from Promega (Madison, WI, USA). Metaphor agarose was obtained from FMC Bioproducts (Rockland, ME, USA). DNA was extracted from lymphocytes using the Wizard genomic DNA isolation kit (Promega). DNA was quantified using UV spectrophotometry, and its purity was determined by the ratio of its absorbance at 260 nm versus 280 nm. [2,2'-<sup>3</sup>H]*N*-OH-ABP (115 mCi/mmol) was prepared from [<sup>3</sup>H]4-nitrobiphenyl (ChemSyn Science Laboratories, Lenexa, KS, USA) by reduction with ammonium polysulfide as described (Thissen *et al.*, 1980). Radiolabelled [ring-<sup>3</sup>H]PhIP (88 mCi/mmol) was purchased from Chem-Syn Science Laboratories. The nitro derivative of PhIP was synthesized according to the method of Grivas

(1988) with the modifications made by Turesky *et al.* (1991).

#### *Platelet and lymphocyte separation*

Individual blood cell components were isolated by centrifugation on discontinuous gradients of Histo-paque-1077 and Histopaque 1119, using a modification of the manufacturer's protocol (Sigma Procedure no. 1119) as described by Frame *et al.* (2000). After separation, platelets were suspended in buffer (0.25 M sucrose, 10 mM triethanolamine, 5 mM 2-mercaptoethanol, pH 7.4) at a concentration of  $1 \times 10^8$  cells/ml. To ensure that the assays reflected activity in platelets and not contaminating cell types, an aliquot was analysed for confirmation of platelet purity, using a Model STKS Coulter counter (Coulter Corp., Irving, TX, USA). Isolated platelets routinely showed negligible contamination with other cell types ( $< 0.08\%$  white blood cells and  $< 0.02\%$  red blood cells). Platelets were used for the preparation of cytosol and the lymphocytes were used for DNA isolation.

#### *SULT1A1 genotype determination*

The polymorphism in the *SULT1A1* gene investigated in these studies consists of a G to A transition that results in an amino acid change (Arg to His, designated *SULT1A1*\*1 and *SULT1A1*\*2, respectively) at residue 213. Detection of the polymorphism was performed according to the method of Ozawa *et al.* (1998). The region of the *SULT1A1* gene flanking the polymorphic base pair was amplified in a PCR reaction using 5'-GGTTGAGGAGTTGGCTCTGC-3' and 5'-ATGAACTCTGGGGGACGGT-3' as forward and reverse primers, respectively. Comparison of the primer pairs, using the NCBI Blast database, showed no significant homology with either *SULT1A2* or *SULT1A3* (Genbank accession numbers U34804 and U20499, respectively). The amplification was performed in 50  $\mu$ l volume containing 100 ng of genomic DNA, 200  $\mu$ M of each dNTP, 1  $\times$  PCR buffer (Promega), 1.5 mM  $MgCl_2$ , 1  $\mu$ M forward and reverse primer, 2.5 U Taq polymerase and 500 ng Taq-Start antibody (Clontech, Palo Alto, CA, USA). After initial denaturation at 95 °C for 4 min, the samples were subjected to 35 cycles of 94 °C for 30 s, 66 °C for 30 s and 72 °C for 90 s, followed by a final extension step of 10 min at 72 °C. The resulting PCR product of 281 bp was then subjected to restriction digest with *Hae*II. Bands were resolved on a 3% Metaphor Agarose gel and visualized by ethidium bromide staining and ultraviolet transillumination. Individuals homozygous for *SULT1A1*\*1 exhibit two bands upon digestion while *SULT1A1*\*2 homozygotes are not cleaved by this enzyme.

#### *Direct sequencing of the SULT1A1 PCR product*

Confirmation of the genotypes was performed by direct sequencing of representative samples using the  $^{33}P$  Thermo Sequenase Radiolabelled Terminator Cycle Sequencing kit (USB Corp., Cleveland, OH, USA) according to the manufacturer's directions. Sequencing reactions were fractionated by electrophoresis on Novex (San Diego, CA, USA) QuickPoint Gels according to the protocol provided by the manufacturer. Sequence bands were detected by autoradiography with overnight exposure of dried gels to X-ray film.

#### *SULT1A1 enzymatic activity assay*

Platelet cytosol for the SULT assays was prepared as previously described (Frame *et al.*, 2000), using supernatant that was subjected to 1 h of 100 000 *g* ultracentrifugation at 4 °C. Protein determinations were performed with the BioRad Protein Assay kit (Hercules, CA, USA) according to the Bradford Method using bovine serum albumin as a standard. Platelet cytosols were assayed for sulfotransferase activity using a simple colorimetric procedure as described by Mulder *et al.* (1977) with the modifications made by Frame *et al.* (2000). Activity was reported as nmol/min/mg protein.

#### *N-OH-ABP and N-OH-PhIP sulfotransferase activity assays*

The assays were carried out as described by Kadlubar *et al.* (1976) with the modifications made by Chou *et al.* (1995a, 1995b). Activity was reported as pmol bound/mg DNA/20 min.

#### *Statistical analysis*

Because there were no statistically significant differences in phenotype between cases and controls in either the breast or colorectal cancer studies, all participants with both genotyping and phenotyping data were included in these analyses ( $n = 279$ ). Phenotypic differences by race and gender were evaluated using Student's *t*-test of means, and non-parametric correlations between genotype and phenotype were evaluated using Spearman's rho. Associations between genotype and phenotype were evaluated using analysis of variance, with phenotype as a continuous variable. Analyses were performed both for the entire dataset and separately by gender and race. Finally, linear regression was used with the natural log of phenotype as the dependent variable to determine the impact of age, race, gender and *SULT1A1* genotype on phenotypic activity.

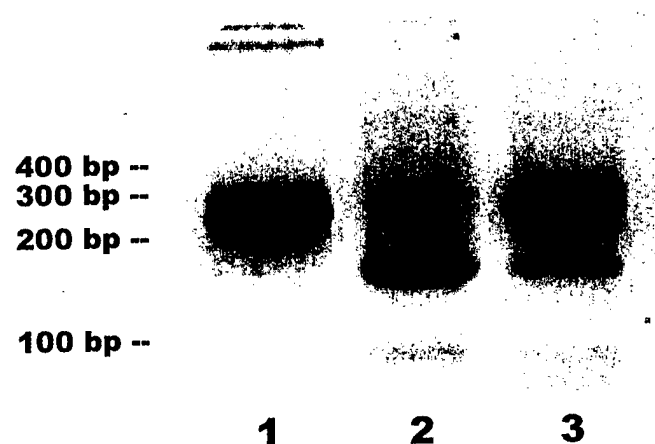
## Results

### SULT1A1 genotyping

Genotyping was performed on DNA from 279 participants; demographic characteristics of the study population are shown in Table 1. The *SULT1A1* G to A transition removes the restriction site for the endonuclease *HaeII*. As shown in Fig. 1, individuals homozygous for the *SULT1A1*\*2 allele do not have

**Table 1.** Demographics of the study population

Age (mean)	60 ± 11
Gender	
Male	96 (34%)
Female	183 (66%)
Race	
Caucasian	239 (86%)
African-American	40 (14%)



**Fig. 1.** Detection of *SULT1A1*\*1/\*2 alleles by restriction fragment length polymorphism analysis. Specific PCR product was generated and digested with *HaeII* as described in Materials and methods. Lane 1, *SULT1A1*\*2/\*2; lane 2, *SULT1A1*\*1/\*2; lane 3, *SULT1A1*\*1/\*1.

the *HaeII* restriction site and consequently, the PCR product is not cleaved (Fig. 1, lane 1). The PCR product from individuals homozygous for *SULT1A1*\*1, however, is cleaved by the enzyme, generating two fragments of approximately 100 and 181 bp (Fig. 1, lane 2). Enzymatic digestion of the PCR product from heterozygotes (*SULT1A1*\*1/\*2) generates one band of 281 bp, along with the 100 and 181 bp fragments (Fig. 1, lane 3). Direct sequencing of the PCR product was performed on representative samples to confirm the genotyping results and to resolve ambiguous results (data not shown). When cases were excluded from the analysis and only controls were considered ( $n = 211$ ), distributions were similar to that for cases and controls combined. *SULT1A1* allele distributions are shown in Table 2 separately for Caucasians ( $n = 240$ ) and African-Americans ( $n = 40$ ). Among Caucasians, 18% of the participants were homozygous for *SULT1A1*\*2 alleles. Although the group size was small, only 10% ( $n = 4$ ) of African-Americans had this genotype.

### Sulfotransferase phenotyping

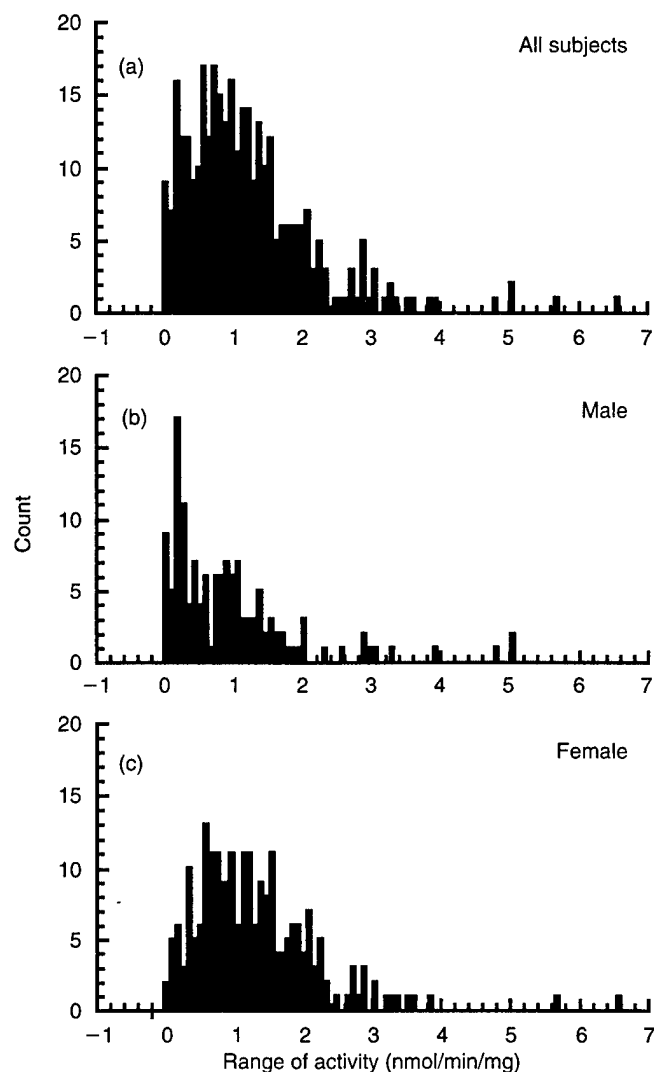
Platelet cytosols from study participants were analysed for SULT activity colorimetrically as described in 'Materials and Methods'. The frequency histograms in Fig. 2 demonstrates the distribution of SULT activity towards 2-naphthol in the study population. Significant ( $P = 0.001$ ) differences in activity were noted between men and women. The mean (SD) activity level for women was 1.28 (0.90) and for men, 0.94 (0.98).

### *SULT1A1* phenotype-genotype correlation

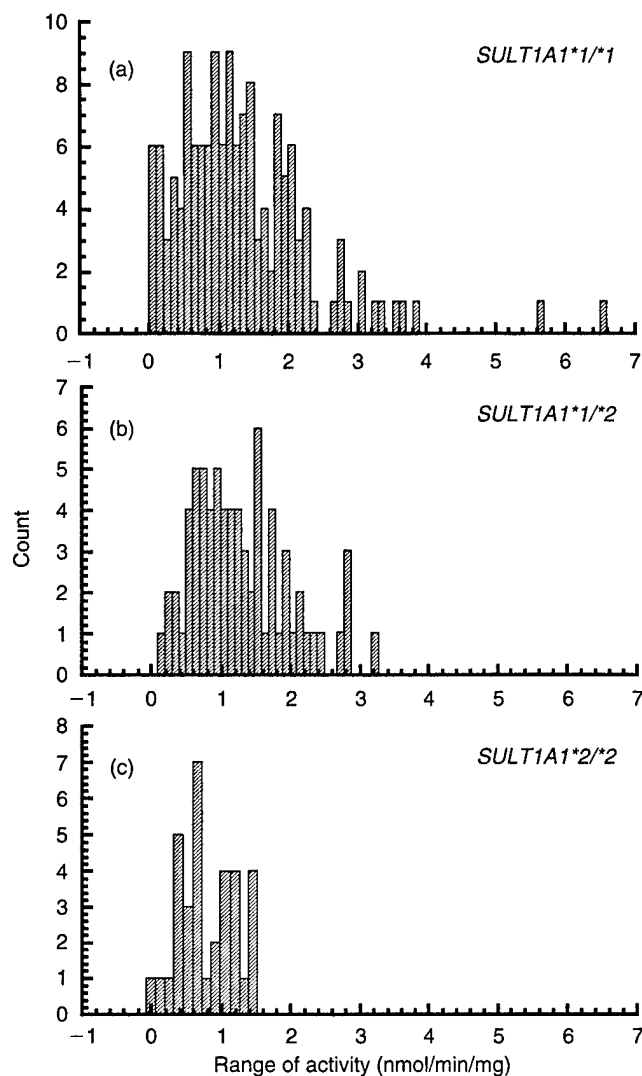
The frequency distribution of activity by genotype is presented in Fig. 3. The correlation between mean levels of SULT activity and *SULT1A1* genotype was 0.29, indicating that genotype predicts less than one-third of SULT phenotype. Using one-way analysis of variance, levels of SULT activity were evaluated by genotype among all participants and separately by race and gender (Table 3). Overall, with each

**Table 2.** *SULT1A1* genotypes in Caucasian and African-American populations

<i>SULT1A1</i> alleles	Caucasian, n (%)	African-American, n (%)
<i>SULT1A1</i> *1/*1	109 (46)	23 (58)
<i>SULT1A1</i> *1/*2	87 (36)	13 (32)
<i>SULT1A1</i> *2/*2	43 (18)	4 (10)
Allele frequencies		
<i>SULT1A1</i> *1	0.64	0.74
<i>SULT1A1</i> *2	0.36	0.26



**Fig. 2.** Frequency histogram analysis of platelet SULT1A1 activity in (a) the entire study population, (b) males only and (c) females only. Activity was assayed as described in the Methods section.



**Fig. 3.** Frequency histogram of genotype dependent SULT1A1 activity in platelet cytosol. (a) *SULT1A1*\*1, (b) *SULT1A1*\*1/\*2 and (c) *SULT1A2*\*2. Activity was assayed as described in the Methods section. The activity for each genotype was calculated and plotted.

**Table 3.** Relationship between mean sulfotransferase activity and *SULT1A1* genotype

	<i>SULT1A1</i> *1/*1	<i>SULT1A1</i> *1/*2	<i>SULT1A1</i> *2/*2
SULT1A1 activity <sup>a</sup>			
All data, $P < 0.001$	( $n = 132$ ) $1.40 \pm 1.06^b$	( $n = 100$ ) $1.18 \pm 0.78$	( $n = 47$ ) $0.74 \pm 0.48$
Females, $P = 0.002$	( $n = 92$ ) $1.44 \pm 0.99$	( $n = 63$ ) $1.34 \pm 0.72$	( $n = 28$ ) $0.78 \pm 0.40$
Males, $P = 0.06$	( $n = 40$ ) $1.32 \pm 1.22$	( $n = 40$ ) $0.91 \pm 0.81$	( $n = 20$ ) $0.68 \pm 0.58$
Caucasians, $P = 0.001$	( $n = 109$ ) $1.36 \pm 1.12$	( $n = 87$ ) $1.12 \pm 0.74$	( $n = 43$ ) $0.76 \pm 0.49$
African-Americans, $P = 0.05$	( $n = 23$ ) $1.60 \pm 0.75$	( $n = 13$ ) $1.58 \pm 0.93$	( $n = 4$ ) $0.53 \pm 0.34$

<sup>a</sup>One-way ANOVA. <sup>b</sup>Activity units are nmol/min/mg protein  $\pm$  SD.

contributing variant allele, there were decreases in levels of activity. When stratifying by gender and race, differences remained in all subgroups, although they were of borderline significance in males. Tukey's post-hoc test revealed that differences were significant primarily between those homozygous for the variant allele and heterozygotes as well as homozygotes for *SULT1A1*\*1/\*1. Although in all strata, heterozygotes had lower activity than those homozygous for the common allele, there were no significant differences in activity between these groups. In a linear regression model, only gender and *SULT1A1* genotype significantly impacted phenotypic activity, with gender having the highest  $\beta$  coefficient (Table 4).

#### *N*-OH-ABP and *N*-OH-PhIP sulfotransferase activity assays

Recombinant *SULT1A1*\*1 and *SULT1A1*\*2 were investigated for their relative abilities to catalyse the binding of *N*-OH-ABP and *N*-OH-PhIP to calf thymus DNA. As shown in Table 5, incubations containing equivalent amounts of expressed *SULT1A1*\*1 and *SULT1A1*\*2 showed that the latter had 10–25% of the activity of the wild-type towards catalysing the PAPS-dependent DNA binding of *N*-OH-ABP and *N*-OH-PhIP.

**Table 4.** Predictors of *SULT1A1* phenotype<sup>a</sup>

Variable	$\beta$ coefficient	P-value
Age	0.0028428	0.61
Race	0.3282531	0.06
Gender	0.5834602	0.000
<i>SULT1A1</i> genotype (*1/*2)	−0.0136802	0.92
<i>SULT1A1</i> genotype (*2/*2)	−0.4673483	0.011

<sup>a</sup>Using a linear regression model, with natural log of phenotype as dependent variable.

**Table 5.** *SULT1A1*-catalysed DNA binding activity in relation to genotype

Substrate	<i>SULT1A1</i> variant	DNA binding (activity <sup>a</sup> , pmol bound/mg DNA/20 min)
<i>N</i> -OH-ABP	<i>SULT1A1</i> *1	5.4
	<i>SULT1A1</i> *2	0.4
<i>N</i> -OH-PhIP	<i>SULT1A1</i> *1	4.6
	<i>SULT1A1</i> *2	1.8

<sup>a</sup>An average of duplicate determinations that were within 10% of each other.

## Discussion

Sulfotransferase activity in platelets has been studied extensively by many investigators. Platelets express both *SULT1A1* and *SULT1A3* and, in the case of *SULT1A1*, the activity correlates to that found in other tissues such as the liver, intestine and brain (Campbell & Weinshilboum, 1986; Young *et al.*, 1985; Sundaram *et al.*, 1989). This correlation, and the easily accessible nature of platelets, has led to their use in population studies. There is substantial interindividual variability in platelet activities; however, it has been reported that there is little intraindividual variability (Anderson & Jackson, 1984; Frame *et al.*, 2000). In contrast to these observations, others have found that platelet *SULT* activity can be influenced by season (Marazziti *et al.*, 1995). These investigators also found a gender difference in the seasonality of activity (Marazziti *et al.*, 1998). *SULT1A1* activity has also been shown to differ by ethnicity (Anderson & Jackson, 1984; Anderson *et al.*, 1988; Kadlubar *et al.*, 1992), with African-Americans having higher *SULT* activity than their Caucasian counterparts.

In addition to catalysing the transfer of the sulfuryl group from PAPS to a phenol acceptor substrate, *SULT1A1* is also capable of transferring the sulfuryl group between two phenols under neutral or acidic conditions (Mulder *et al.*, 1977). Incubation of platelet cytosol with both 2-naphthol and *p*-nitrophenyl sulfate in the presence of micromolar amounts of PAPS generates 2-naphthyl sulfate and *p*-nitrophenol (PNP). PNP can be quantified colorimetrically by changes in absorbance at 405 nm under alkaline conditions. Generation of PNP has been shown to correlate directly with the formation of 2-naphthyl sulfate (Mulder *et al.*, 1977). While *SULT1A1* and *SULT1A3* are both capable of sulfating 2-naphthol, assays using recombinant enzymes demonstrate that the contribution of *SULT1A3* to the assay is negligible. To determine if the highly homologous *SULT1A2* is contributing to the enzymatic assay, analysis of mRNA from platelets was performed. Platelets contained transcript for both *SULT1A1* and *SULT1A3*, but message for *SULT1A2* was not detected (Frame *et al.*, 2000). Therefore, with this particular assay, the activity measured in platelet cytosol is due solely to *SULT1A1*.

Inheritance studies performed in the late 1980s indicated that platelet *SULT* activity was influenced by genetic polymorphisms (Price *et al.*, 1988, 1989) but, at that time, the molecular basis of the polymorphisms was unknown. Recently, polymorphisms in *SULT1A1* have been identified (Raftogianis *et al.*, 1997; Ozawa *et al.*, 1998). The variant, *SULT1A1*\*2,



was associated with low SULT1A1 activity and thermostability, although the number of samples with phenotype data available was small (Raftogianis *et al.*, 1997).

In this study, we sought to demonstrate a correlation between genotype and platelet phenotype using a microtiter plate assay for platelet SULT1A1 activity, along with genotyping for the *SULT1A1*\*1/*1A1*\*2 polymorphism. The allele frequencies were slightly different from those published previously (Raftogianis *et al.*, 1997; Ozawa *et al.*, 1998; Coughtrie *et al.*, 1999), possibly due to numbers genotyped and the nature of the population surveyed. A distinct difference in the allele frequency between African-Americans and Caucasians was observed, with African-Americans being less likely to be homozygous for the *SULT1A1*\*2 allele (10% of African-Americans compared to 18% Caucasians). This is in contrast to the allele frequencies demonstrated by Coughtrie *et al.* (1999) who found no statistically significant differences in allele frequencies between a Nigerian and a Caucasian population. This disparity is possibly due to the intrinsic differences between the populations studied (Nigerian versus African-American) or to the small number of African-Americans in the present study. When phenotype was compared, however, African-Americans consistently exhibited higher activity levels for each genotype than Caucasians (Table 3). This is in agreement with findings by other investigators who have demonstrated higher SULT activity in platelets from African-Americans versus Caucasians. When African-American males were compared with Caucasian males, overall activity was higher, thus excluding the possibility of the data reflecting gender differences. It must be noted, however, that the number of African-Americans in this study is very small, so these observations are preliminary.

There was also a difference in SULT1A1 activity between males and females in this study, with females having significantly higher levels than males. This finding is in contrast to other studies, which either did not see significant gender differences (Price *et al.*, 1989) or found that, in certain ethnic populations, males possess higher SULT1A1 activity (Brittelli *et al.*, 1999). The mechanism of the elevation in SULT1A1 activity in females has not been elucidated. Hormonal regulation of another member of the phenol sulfotransferase subfamily, SULT1E, by progesterone has been demonstrated (Falany & Falany, 1996a, 1996b). Since both platelets and their parent cell, the megakaryocyte, contain oestrogen and androgen receptors (Tarantino *et al.*, 1994; Khetawat *et al.*, 2000), it is possible that the difference in activity between males and females could

have a hormonal basis. Although sample preparation has been standardized so that every blood specimen is prepared in the same manner, it is also possible that some of the differences in activity could be due to the increased thermolability of the enzyme in individuals expressing the homozygous *SULT1A1*\*2.

There was a dose-dependent effect of genotype on platelet SULT phenotype throughout this study. Individuals possessing the *SULT1A1*\*2/*1*\*2 allele consistently displayed lower platelet activity, while heterozygous individuals tended to have intermediate activity and those homozygous for *SULT1A1*\*1 exhibited the highest overall platelet SULT activity. The biological role, if any, of sulfation by platelet isoforms is unknown at the present time. However, the correlation of platelet SULT1A1 activity with the activity in other tissues provides the opportunity to examine the relationship between genetic polymorphisms and phenotype independently of the contribution of other SULT isoforms to activity levels. This study demonstrates a statistically significant correlation between genotype and platelet phenotype. However, analysis of variance indicates that this genotype accounts for less than 30% of the phenotypic variation observed. Moreover, probit analysis of each genotype indicates that the stratified phenotype is not normally distributed and that there are likely other genetic determinants of SULT1A1 activity.

Studies using recombinant *SULT1A1* allelic variants revealed that the wild-type, *SULT1A1*\*1 catalysed the binding of proximate carcinogens much more efficiently than did the *SULT1A1*\*2 variant. This is in agreement with the proposed role of SULT1A1 in the activation of heterocyclic amines to DNA binding species, which is one of the steps involved in tumour formation. Given the potential role of SULT1A1 in chemical carcinogenesis, further investigation of this genotype-phenotype interaction in the context of a case-control study is under way in our laboratory.

### Acknowledgements

This work was supported by Department of Defense Breast Cancer Research Program (DAMB17-98-I-ABDO), the Food and Drug Administration's Office of Women's Health, NIH grants CA55751, CA58697, EPA grant R825280 and NIA grant AG15722. We also wish to thank Candee Teitel and Bridgette Green for carrying out the DNA binding assays.

### References

- Abu-Zeid M, Yamazoe Y, Kato R. Sulfotransferase-mediated DNA binding of N-hydroxyarylamines (amide) in liver cytosols

- from human and experimental animals. *Carcinogenesis* 1992; **13**:1307-1314.
- Anderson RJ, Jackson BL. Human platelet phenol sulfotransferase: stability of two forms of the enzyme with time and presence of a racial difference. *Clin Chim Acta* 1984; **138**:185-196.
- Anderson RJ, Jackson BL, Liebenritt DK. Human platelet thermostable phenol sulfotransferase from blacks and whites: biochemical properties and variations in thermal stability. *J Lab Clin Med* 1988; **112**:773-783.
- Anderson RJ, Garcia MJ, Liebenritt DK, Kay HD. Localization of human blood phenol sulfotransferase activities: novel detection of the thermostable enzyme in granulocytes. *J Lab Clin Med* 1991; **118**:500-509.
- Anderson KE, Hammons GJ, Kadlubar FF, Potter JD, Kaderlik KR, Ilett KF, et al. Metabolic activation of aromatic amines by human pancreas. *Carcinogenesis* 1997; **18**:1085-1092.
- Anderson RJ, Kudlacek PE, Clemens DL. Sulfation of minoxidil by multiple human cytosolic sulfotransferases. *Chem Biol Interact* 1998; **109**:53-67.
- Beutler E, Gelbart T, Demina A. Racial variability in the UDP-glucuronosyltransferase 1 (UGT1A1) promoter: a balanced polymorphism for regulation of bilirubin metabolism? *Proc Natl Acad Sci USA* 1998; **95**:8170-8174.
- Brittelli A, De Santi C, Raunio H, Pelkonen O, Rossi G, Pacifici GM. Interethnic and interindividual variabilities of platelet sulfotransferases activity in Italians and Finns. *Eur J Clin Pharmacol* 1999; **55**:691-695.
- Campbell N, Weinshilboum R. Human phenol sulfotransferase (PST): correlation of liver and platelet activities. *Can Soc Clin Invest* 1986; **9**:A14.
- Cappiello M, Franchi M, Rane A, Pacifici GM. Sulphotransferase and its substrate: adenosine-3'-phosphate-5'-phosphosulphate in human fetal liver and placenta. *Dev Pharmacol Ther* 1990; **14**:62-65.
- Chou HC, Lang NP, Kadlubar FF. Metabolic activation of N-hydroxy arylamines and N-hydroxy heterocyclic amines by human sulfotransferase(s). *Cancer Res* 1995a; **55**:525-529.
- Chou HC, Lang NP, Kadlubar FF. Metabolic activation of the N-hydroxy derivative of the carcinogen 4-aminobiphenyl by human tissue sulfotransferases. *Carcinogenesis* 1995b; **16**:413-417.
- Coughtrie MW. Sulphation catalysed by the human cytosolic sulphotransferases - chemical defence or molecular terrorism? *Hum Exp Toxicol* 1996; **15**:547-555.
- Coughtrie MW, Gilissen RA, Shek B, Strange RC, Fryer AA, Jones PW, Bamber DE. Phenol sulphotransferase SULT1A1 polymorphism: molecular diagnosis and allele frequencies in Caucasian and African populations. *Biochem J* 1999; **337**:45-49.
- Critchley JA, Nimmo GR, Gregson CA, Woolhouse NM, Prescott LF. Inter-subject and ethnic differences in paracetamol metabolism. *Br J Clin Pharmacol* 1986; **22**:649-657.
- Falany JL, Falany CN. Expression of cytosolic sulfotransferases in normal mammary epithelial cells and breast cancer cell lines. *Cancer Res* 1996a; **56**:1551-1555.
- Falany JL, Falany CN. Regulation of estrogen sulfotransferase in human endometrial adenocarcinoma cells by progesterone. *Endocrinology* 1996b; **137**:1395-1401.
- Frame L, Ozawa S, Nowell S, Chou H-C, DeLongchamp R, Lang N, Kadlubar F. A simple colorimetric assay for phenotyping the major human thermostable phenol sulfotransferase (SULT1A1) using platelet cytosols. *Drug Metabol Dispos* 2000; **28**:1063-1068.
- Gilissen RA, Bamforth KJ, Stavenuiter JF, Coughtrie MW, Meerman JH. Sulfation of aromatic hydroxamic acids and hydroxylamines by multiple forms of human liver sulfotransferases. *Carcinogenesis* 1994; **15**:39-45.
- Glatt H. Sulfation and sulfotransferases 4: bioactivation of mutagens via sulfation. *FASEB J* 1997; **11**:314-321.
- Gonzalez FJ. Genetic polymorphism and cancer susceptibility: fourteenth Sapporo Cancer Seminar. *Cancer Res* 1995; **55**:710-715.
- Grivas S. Synthesis of 3,8-dimethyl-2-nitro-<sup>3</sup>H-imidazo[4,5-f]quinoxaline, the 2-nitro analogue of the food carcinogen MeIQx. *J Chem Res* 1988; **5**:84.
- Hart RF, Renskers KJ, Nelson EB, Roth JA. Localization and characterization of phenol sulfotransferase in human platelets. *Life Sci* 1979; **24**:125-130.
- Her C, Aksoy IA, Kimura S, Brandriff BF, Wasmuth JJ, Weinshilboum RM. Human estrogen sulfotransferase gene (STE): cloning, structure, and chromosomal localization. *Genomics* 1995; **29**:16-23.
- Her C, Raftogianis R, Weinshilboum RM. Human phenol sulfotransferase STP2 gene: molecular cloning, structural characterization, and chromosomal localization. *Genomics* 1996; **33**:409-420.
- Her C, Kaur GP, Athwal RS, Weinshilboum RM. Human sulfotransferase SULT1C1: cDNA cloning, tissue-specific expression, and chromosomal localization. *Genomics* 1997; **41**:467-470.
- Her C, Wood TC, Eichler EE, Mohrenweiser HW, Ramagli LS, Siciliano MJ, Weinshilboum RM. Human hydroxysteroid sulfotransferase SULT2B1: two enzymes encoded by a single chromosome 19 gene. *Genomics* 1998; **53**:284-295.
- Hume R, Barker EV, Coughtrie MW. Differential expression and immunohistochemical localisation of the phenol and hydroxysteroid sulphotransferase enzyme families in the developing lung. *Histochem Cell Biol* 1996; **105**:147-152.
- Jakoby WB, Ziegler DM. The enzymes of detoxication. *J Biol Chem* 1990; **265**:20715-20718.
- Kadlubar FF, Miller JA, Miller EC. Hepatic metabolism of N-hydroxy-N-methyl-4-aminoazobenzene and other N-hydroxy arylamines to reactive sulfuric acid esters. *Cancer Res* 1976; **36**:2350-2359.
- Kadlubar FF, Butler MA, Kaderlik KR, Chou HC, Lang NP. Polymorphisms for aromatic amine metabolism in humans: relevance for human carcinogenesis. *Environ Health Perspect* 1992; **98**:69-74.
- Kalow W. Interethnic variation of drug metabolism. *Trends Pharmacol Sci* 1991; **12**:102-107.
- Khetawat G, Faraday N, Nealen ML, Vijayan KV, Bolton E, Noga SJ, Bray PF. Human megakaryocytes and platelets contain the estrogen receptor beta and androgen receptor (AR): testosterone regulates AR expression. *Blood* 2000; **95**:2289-2296.
- Kudlacek PE, Anderson RJ, Liebenritt DK, Johnson GA, Huerter CJ. Human skin and platelet minoxidil sulfotransferase activities: biochemical properties, correlations and contribution of thermolabile phenol sulfotransferase. *J Pharmacol Exp Ther* 1995; **273**:582-590.
- Llerena A, Cobaleda J, Martinez C, Benitez J. Interethnic differences in drug metabolism: influence of genetic and environmental factors on debrisoquine hydroxylation phenotype. *Eur J Drug Metab Pharmacokinet* 1996; **21**:129-138.
- Massaad L, de Waziers I, Ribrag V, Janot F, Beaune PH, Morizet J, et al. Comparison of mouse and human colon tumors with regard to phase I and phase II drug-metabolizing enzyme systems. *Cancer Res* 1992; **52**:6567-6575.
- Marazziti D, Palego L, Mazzanti C, Silvestri S, Cassano GB.

- Human platelet sulfotransferase shows seasonal rhythms. *Chronobiol Int* 1995; **12**:100-105.
- Marazziti D, Palego L, Rossi A, Cassano GB. Gender-related seasonality of human platelet phenolsulfotransferase activity. *Neuropsychobiology* 1998; **38**:1-5.
- Miller JA. Sulfonation in chemical carcinogenesis - history and present status. *Chem Biol Interact* 1994; **92**:329-341.
- Mulder GJ, Hinson JA, Gillette JR. Generation of reactive metabolites of N-hydroxy-phenacetin by glucuronidation and sulfation. *Biochem Pharmacol* 1977; **26**:189-196.
- Ozawa S, Chou HC, Kadlubar FF, Nagata K, Yamazoe Y, Kato R. Activation of 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-b] pyridine by cDNA-expressed human and rat arylsulfotransferases. *Jpn J Cancer Res* 1994; **85**:1220-1228.
- Ozawa S, Tang YM, Yamazoe Y, Kato R, Lang NP, Kadlubar FF. Genetic polymorphisms in human liver phenol sulfotransferases involved in the bioactivation of N-hydroxy derivatives of carcinogenic arylamines and heterocyclic amines. *Chem Biol Interact* 1998; **109**:237-248.
- Price RA, Cox NJ, Spielman RS, Van Loon JA, Maidak BL, Weinshilboum RM. Inheritance of human platelet thermolabile phenol sulfotransferase (TL PST) activity. *Genet Epidemiol* 1988; **5**:1-15.
- Price RA, Spielman RS, Lucena AL, Van Loon JA, Maidak BL, Weinshilboum RM. Genetic polymorphism for human platelet thermostable phenol sulfotransferase (TS PST) activity. *Genetics* 1989; **122**:905-914.
- Raftogianis RB, Her C, Weinshilboum RM. Human phenol sulfotransferase pharmacogenetics: STP1 gene cloning and structural characterization. *Pharmacogenetics* 1996; **6**:473-487.
- Raftogianis RB, Wood TC, Otterness DM, Van Loon JA, Weinshilboum RM. Phenol sulfotransferase pharmacogenetics in humans: association of common SULT1A1 alleles with TS PST phenotype. *Biochem Biophys Res Commun* 1997; **239**:298-304.
- Reveley AM, Bonham Carter SM, Reveley MA, Sandler M. A genetic study of platelet phenolsulphotransferase activity in normal and schizophrenic twins. *J Psychiatr Res* 1982; **17**:303-307.
- Sakakibara Y, Yanagisawa K, Katafuchi J, Ringer DP, Takami Y, Nakayama T, et al. Molecular cloning, expression, and characterization of novel human SULT1C sulfotransferases that catalyze the sulfonation of N-hydroxy-2-acetylaminofluorene. *J Biol Chem* 1998; **273**:33929-33935.
- Stephens EA, Taylor JA, Kaplan N, Yang CH, Hsieh LL, Lucier GW, Bell DA. Ethnic variation in the CYP2E1 gene: polymorphism analysis of 695 African-Americans, European-Americans and Taiwanese. *Pharmacogenetics* 1994; **4**:185-192.
- Sundaram RS, Van Loon JA, Tucker R, Weinshilboum RM. Sulfation pharmacogenetics: correlation of human platelet and small intestinal phenol sulfotransferase. *Clin Pharmacol Ther* 1989; **46**:501-509.
- Tarantino MD, Kunicki TJ, Nugent DJ. The estrogen receptor is present in human megakaryocytes. *Ann NY Acad Sci* 1994; **714**:293-296.
- Thissen M, Roth R, Duncan W. Convenient synthesis of selected <sup>14</sup>C- and <sup>3</sup>H-labeled aromatic hydroxylamines. *Org Prep Proced* 1980; **12**:337-344.
- Turesky RJ, Lang NP, Butler MA, Teitel CH, Kadlubar FF. Metabolic activation of carcinogenic heterocyclic aromatic amines by human liver and colon. *Carcinogenesis* 1991; **12**:1839-1845.
- Walker AH, Jaffe JM, Gunasegaram S, Cummings SA, Huang CS, Chern HD, et al. Characterization of an allelic variant in the nifedipine-specific element of CYP3A4: ethnic distribution and implications for prostate cancer risk. Mutations in brief no. 191. Online. *Hum Mutat* 1998; **12**:289.
- Weinshilboum RM, Otterness DM, Aksoy IA, Wood TC, Her C, Raftogianis RB. Sulfation and sulfotransferases 1: sulfotransferase molecular biology: cDNAs and genes. *FASEB J* 1997; **11**:3-14.
- Windmill KF, McKinnon RA, Zhu X, Gaedigk A, Grant DM, McManus ME. The role of xenobiotic metabolizing enzymes in arylamine toxicity and carcinogenesis: functional and localization studies. *Mutat Res* 1997; **376**:153-160.
- Wood TC, Aksoy IA, Aksoy S, Weinshilboum RM. Human liver thermolabile phenol sulfotransferase: cDNA cloning, expression and characterization. *Biochem Biophys Res Commun* 1994; **198**:1119-1127.
- Wood TC, Her C, Aksoy I, Otterness DM, Weinshilboum RM. Human dehydroepiandrosterone sulfotransferase pharmacogenetics: quantitative Western analysis and gene sequence polymorphisms. *J Steroid Biochem Mol Biol* 1996; **59**:467-478.
- Young WF Jr, Laws ER Jr, Sharbrough FW, Weinshilboum RM. Human phenol sulfotransferase: correlation of brain and platelet activities. *J Neurochem* 1985; **44**:1131-1137.
- Zou JY, Petney R, Roth JA. Immunohistochemical detection of phenol sulfotransferase-containing neurons in human brain. *J Neurochem* 1990; **55**:1154-1158.